Family-level leaf nitrogen and phosphorus stoichiometry of global terrestrial plants

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Abstract: Leaf nitrogen (N) and phosphorus (P) concentrations are critical for photosynthesis, growth, reproduction and other ecological processes of plants. Previous studies on large-scale biogeographic patterns of leaf N and P stoichiometric relationships were mostly conducted using data pooled across taxa, while family/genus-level analyses are rarely reported. Here, we examined global patterns of family-specific leaf N and P stoichiometry using a global data set of 12,716 paired leaf N and P records which includes 204 families, 1,305 genera, and 3,420 species. After determining the minimum size of samples (i.e., 35 records), we analyzed leaf N and P concentrations, N:P ratios and N P scaling relationships of plants for 62 families with 11,440 records. The numeric values of leaf N and P stoichiometry varied significantly across families and showed diverse trends along gradients of mean annual temperature (MAT) and mean annual precipitation (MAP). The leaf N and P concentrations and N:P ratios of 62 families ranged from 6.11 to 30.30 mg g−1, 0.27 to 2.17 mg g−1, and 10.20 to 35.40, respectively. Approximately 1/3–1/2 of the families (22–35 of 62) showed a decrease in leaf N and P concentrations and N:P ratios with increasing MAT or MAP, while the remainder either did not show a significant trend or presented the opposite pattern. Family-specific leaf N P scaling exponents did not converge to a certain empirical value, with a range of 0.307–0.991 for 54 out of 62 families which indicated a significant N P scaling relationship. Our results for the first time revealed large variation in the family-level leaf N and P stoichiometry of global terrestrial plants and that the stoichiometric relationships for at least one-third of the families were not consistent with the global trends reported previously. The numeric values of the family-specific leaf N and P stoichiometry documented in the current study provide critical synthetic parameters for biogeographic modeling and for further studies on the physiological and ecological mechanisms underlying the nutrient use strategies of plants from different phylogenetic taxa.

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Abstract:

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1 INTRODUCTION

Nitrogen (N) and phosphorus (P) are essential components of basic cell structure of higher plants, especially playing pivotal roles in the synthesis and transformation of protein and nucleic acids (Garten, 1976). N and P concentrations and their stoichiometric balances of leaves, the fundamental tissues for photosynthesis of higher plants, determine the whole plants' photosynthate, growth, reproduction and other functional traits, and further influence soil-plant nutrient cycling, community biodiversity dynamics, vegetation productivity and ecosystem succession (Asner et al., 1997; Niklas et al., 2005; Reich et al., 2009; Sullivan et al., 2014; Tang et al., 2018).

As key properties of leaf economic spectrum (i.e. LES), leaf N and P concentrations generally show strong correlation in their biochemical functioning (Wright et al., 2004; Ågren, 2008). Previous studies have figured out the broad-scale leaf N and P stoichiometric patterns and proposed several hypothetical theories through their exploration of nutrient stoichiometry from regional to global scales. For example, Reich et al. (2004) generalized the global patterns of higher plant leaf N and P stoichiometry across latitudinal and temperature gradients (e.g. leaf N and P increase from tropics to the midlatitudes and keep stable or decrease at higher latitudes, and N:P ratios increases with mean temperature), and further tested the temperature-plant physiological hypotheses and biogeochemical hypotheses. Using data of national terrestrial plants, Han et al. (2005) reported that the overall leaf N:P ratios of China's flora was higher than the global averages even though leaf N and P showed consistent trends along latitudinal gradients, which was probably a result of limited soil P availability in China according to the soil substrate age hypothesis (Carnicer et al., 2014). At regional level, specific species (e.g. *Picea abies* and *Artemisa* species) also showed divergent geographic patterns along longitudinal (i.e. MAP), latitudinal (i.e. MAT) and altitudinal gradients (Kang et al., 2011; Yang et al., 2015). In terms of different growth forms, the relative growth rate hypothesis (i.e. GRH) has attracted tremendous interest and been prevalently used in explaining the variations of elemental stoichiometry across growth forms (Chapin et al., 1986; Sterner and Elser, 2002; Tian et al., 2018). For example, woody plants, compared to herbaceous plants, generally show lower N and P concentrations for their slower growth rates and effective physiological strategies for nutrient conservation (e.g. improving nutrient use efficiency through their intrinsic nutrient resorption and transformation strategies) (Elser, et al., 2010; McGroddy et al., 2008; Brant and Chen, 2015).

Leaf N:P ratios and N~P scaling relationships are two indicators of the coupling relationships between leaf N and P concentrations. The later one can be quantified via a stoichiometric scaling relationship described by a power function as $N = \beta P^{\alpha}$, where α and β represent the scaling exponent and the intercept (i.e. normalization constant) of the log-log linear leaf N concentration vs. P concentration regression curve, respectively (Wright et al., 2004; Niklas, 2006; Tian et al., 2018). When α equals to 1, the numeric value of β is leaf N:P ratio because of the isometric scaling relationship. Alternatively, $\alpha < 1$ indicates a faster change of leaf P concentration in proportion to leaf N concentration while $\alpha > 1$ indicates the opposite case (Niklas et al., 2005; Kerkhoff et al., 2006; McGroddy et al., 2008).

In various ecosystems, leaf N:P ratios were widely interpreted as indicators of environmental N and P relative availabilities. Despite their shortages and potential high risks (Yan et al., 2017), empirical values of leaf N:P ratios proposed in previous studies at community or vegetation levels have been commonly used as thresholds for determining N or P deficiency (e.g. N:P ratios <14 and >16 or <10 and >20 were used as thresholds for assessing N and P limitation, respectively) (Koerselman and Meuleman, 1996; Güsewell, 2004). Leaf N~P scaling relationships were also interpreted as plants' physiological strategies resulted from their evolutionary adaptation to environmental nutrient availabilities although the underlying mechanisms remains unclear. For example, the 2/3- and 3/4-power laws obtained from pooled data have been used to ecological theories and prediction models as important plant functional traits and parameters (e.g. biogeochemical models; Carbon, N and P cycling models; Nutrients-vegetation productivity prediction models; The relative growth rate hypothesis and metabolic models; Stoichiometric homeostasis hypothesis; Metabolic scaling theory) (Wright et al., 2005; Allen and Gillooly, 2009; Elser et al., 2010; Carnicer et al., 2014; Tang et al., 2018).

Overall, previous studies were mainly focused on the large-scale patterns of leaf N and P stoichiometric relationships across geographic gradients in relation to climatic and environmental factors (Wright et al., 2004; Reich et al., 2004; Chen et al., 2013; Hao et al., 2015; Zhang et al., 2018). However, whether the general leaf N and P stoichiometric relationship hold at more specific scales and could be used as constant plant trait in related models has attracted researchers' attention (Messier et al. 2016; Tian et al., 2018). For example, our recent study has demonstrated that leaf N and P scaling exponent vary significantly across different functional groups, latitudinal zones, ecological biomes and specific sites (Tian et al., 2018), which resonated with Messier et al. (2016)'s argument that LES may not hold or show large variation at smaller scales.

Likewise, phylogenetic taxa represent a kind of scale. Some researchers have reported the phylogenetic signals in leaf N and P stoichiometry (e.g. Thompson et al., 1997; Kerkhoff et al., 2006; Peñuelas et al., 2010), especially at family- and subfamily-levels (Zhang et al., 2012; Hao et al., 2015). Hence, it provides us an alternative approach to verify previous theories and hypothesis of leaf N and P stoichiometric relationships on the family-levels (White et al., 2012; Zhang et al., 2012), which also has possibilities to fulfill the considerable requirements for detail parameters in plant physiological and ecosystem functioning prediction models (Sterner

and Elser, 2002; Osnas et al., 2013; Tang et al., 2018). However, no comprehensive study to date has comprehensively conducted considering leaf N and P stochiometric relationships on family-levels.

Our study aims to fill these gaps by providing to date the largest global leaf N and P concentration dataset and analyzing leaf N and P stoichiometric relationships on family-level of higher plants. To do this, we calculated leaf N and P stoichiometric values of specific families and examined their relationships with climatic factors. Our objectives are to test the following questions: (1) how higher plants leaf N and P stochiometric relationships change on the family-levels ? (2) how their geographical patterns change along MAT and MAP ? (3) do leaf N~P scaling exponents of specific families converge to an empirical value (i.e. whether the global-level leaf N~P scaling exponent hold on the family-level) ?

2 MATERIALS AND METHODS

2.1 Dataset

We established a global data set of paired leaf N and P records following strict principles. Records of paired N and P concentrations of green leaves with detailed location information were compiled in the data set. Unpaired and mismatched leaf N-P records, records without site information, plants cultivated in greenhouse or plantations and duplicated records were directly excluded. Finally, 4,212 records from the TRY data set (https://www.try-db.org) (Kattge et al., 2011) and 8,509 records from previous studies and our own field sampling were adopted to the data set (Tian et al., 2018). All the records of plant samples were collected in their growing season and leaf N and P concentration were detected on the mass unit. The phylogenetic taxa information of the total 12,721 records in our data set were then identified using Flora of China (http://frps.eflora.cn/), including 204 families, 1,305 genus and 3420 species.

2.2 Statistic analysis

To avoid potential error risks induced from the sample sizes of specific families, we firstly used Monte Carlo methods and randomly sampled certain numbers of pairwise leaf N and P records in our data set from 5 to 10,000 at 1000 iterations, respectively. We then calculated the statistics including geometric means, ranges, coefficient of variance (CV), standard error (SE) of leaf N and P concentrations, N:P ratios and N~P scaling relationships based on the randomly sampled

records. During these analysis, we used the original values of the individual paired records. For the statistics of N~P scaling relationships, we used the reduced major axis (RMA) regression (Warton et al., 2006) after log10-transforming the original values of N and P concentration. Additionally, we used a likelihood ratio test to evaluate the differences between RMA regression exponents of each family and the empirical values (i.e. 2/3 and 3/4).

During the performing of Monte Carlo sampling, we found that the variation of leaf N and P stoichiometric statistics became smaller with the increase of sample size. When the sample sizes were more than 35, the CVs of their leaf N and P concentrations and N:P ratios were below 10% (Table S1, Figures S1 and S2). Similarly, the frequencies of significant N~P scaling relationships in 1000 iterations increased with sample size and were higher than 90% with CV values below 15% when the sample sizes were more than 35 (Table S2, Figure S3a and 3b). Hence, we chose all the families with at least 35 individual records from the dataset for further statistical analysis. Additionally, we explored the biogeographic patterns of leaf N and P stoichiometry of each family along MAT and MAP, respectively, using a linear model. The data of MAT and MAP were extracted from WorldClim (http://www.worldclim.org/). All statistical analyses were performed in the R 3.5.1 software (R core Team, 2018).

3 RESULTS

3.1 Leaf N and P stoichiometry on family-level

There were 62 families with at least 35 individual records in our dataset. Their geometric means of N and P concentrations and N:P ratios ranged from 6.11 to 30.30 mg g^{-1}, from 0.27 to 2.17 mg g^{-1}, from 10.20 to 35.40, respectively (Table 1).

Specifically, leaf N and P concentrations of Proteaceae and Gleicheniaceae were the lowest among 62 families (i.e. leaf N concentrations were 6.11 and 10.08 mg g^{-1} and leaf P concentrations were 0.27 and 0.43 mg g^{-1}, respectively). Leaf N concentrations of Myrtaceae, Cupressaceae, Theaceae and Pinaceae were lower than other families (i.e. from 10.89 to 13.52 mg g^{-1}). Moreover, leaf P concentrations of Myricaceae, Myrtaceae, Lecythidaceae, Sapotaceae, Symplocaceae, Melastomataceae and Theaceae were low (i.e. from 0.48 to 0.68 mg g^{-1}).

Comparatively, leaf N concentrations of Rutaceae, Chenopodiaceae, Ulmaceae, Liliaceae, Umbelliferae, Tiliaceae, Ranunculaceae, Leguminosae, Polygonaceae, Zygophyllaceae and

Elaeagnaceae were high (i.e. from 23.18 to 30.30 mg g^{-1}). Leaf P concentrations of Labiatae, Salicaceae, Liliaceae, Tiliaceae, Aceraceae, Ranunculaceae, Polygonaceae and Umbelliferae were high (i.e. from 1.80 to 2.12 mg g^{-1}).

We additionally found that leaf N:P ratios of Cupressaceae, Pinaceae, Plantaginaceae, Umbelliferae, Compositae, Salicaceae, Aceraceae and Labiatae were low (i.e. from 10.5 to 12.2), while the N:P ratios of Symplocaceae, Melastomataceae, Proteaceae, Leguminosae, Burseraceae, Gleicheniaceae, Sapotaceae, Lecythidaceae and Myricaceae were high (i.e. from 22.2 to 35.4).

3.2 Biogeographic patterns of leaf N and P stoichiometry on family-level

62 families showed large variation in their location distribution. Their N and P concentrations and N:P ratios also showed divergent biogeographic patterns along MAT and MAP, respectively (Table S3, Figure 1).

Specifically, in terms of leaf N concentrations, 33 families declined and 4 families increased with increasing MAT, 22 families declined and 8 families increased with increasing MAP, while 22 families showed no significant change with MAT and MAP. For leaf P concentrations, 29 families declined and 3 families increased with increasing MAT, 35 families declined and 7 families increased with increasing MAP, whereas 30 and 20 families showed no significant change with MAT and MAP, respectively. For leaf N:P ratios, 29 families increased and 3 families decreased with increasing MAT, 24 families declined and 2 families increased with increasing MAP, whereas 30 and 36 families showed no significant change with MAT and MAP, respectively.

3.3 Leaf N~P scaling relationship on family-level

54 of the 62 families showed significant leaf N and P scaling relationships (Tables 2 and S3) and their scaling exponents ranged from 0.307 to 0.991 with a geometric mean of 0.633 (Table 2, Figure 2), while the other 8 families' leaf N and P concentrations were not statistically significantly correlated.

Moreover, when comparing 54 families' leaf N~P scaling exponents with empirical numeric values (i.e. 2/3 and 3/4), 23 families showed significant difference with 2/3 (i.e. 13 families < 2/3 and 10 families > 2/3) whereas 31 families showed no significant difference with 2/3.

Similarly, 30 families were significantly different from 3/4 (i.e. 27 families < 3/4 and 3 families > 3/4) while 24 families showed no significant difference with 3/4. Meanwhile, 17 families' leaf N~P scaling exponents covered both 2/3 and 3/4 (Table 2).

4 DISCUSSION

4.1 Variations in leaf N and P stoichiometry on family-level

Leaf N and P concentrations and their stoichiometric relationships closely correlated with plant growth and performance (Garten, 1976; Nielsen et al., 1996; Elser et al., 2000, 2010). Variation in leaf N and P concentrations on family-level reflected the joint influences of interior genetic properties and extrinsic environmental factors (Kerkhoff et al., 2006; Ågren and Weih, 2012). The former included leaf morphology, leaf lifespan, leaf age, nutrient storage and allocation among tissues, plant growth rate, stoichiometric homeostasis and plasticity and other physiological processes (Chapin et al., 1986; Elser et al., 2010; Sardans et al., 2012; Sistla et al., 2015). The latter mainly covered climatic conditions, soil physiochemical properties and nutrient availabilities (Vitousek and Turner, 1995; Zhang et al., 2012; Hao et al., 2015).

As a result of divergent nutrient use strategies and specific leaf structures and metabolic functions, the variations in leaf N and P stoichiometry on family-level revealed plants' adaptation to biotic and abiotic factors during their evolution (Güsewell, 2004; Sardans and Peñuelas, 2014； Delgado et al., 2014; Sardans et al., 2016). For example, leaf N and P concentrations of Proteaceae, Gleicheniaceae and Myrtaceae families were comparatively low with high N:P ratios among 62 families. Gymnosperms including Cupressaceae and Pinaceae families showed low N concentrations and N:P ratios. These results were consistent with previous studies on regional scales (e.g. Fould, 1993; Thompson et al., 1997). In general, as most plants of these families were oligotrophic pioneer species in the early stage of community succession in nutrient-poor soils, they developed high tolerance to environmental stress and effective nutrient use strategies (Page et al., 2002; Lamber et al., 2008; Fujita et al., 2013; Sardans et al., 2016). Especially, the Proteaceae family that distributed in severe P limited environment evolved adaptive structures and functions, such as enhanced efficiencies of P resorption, P transformation and reallocation across different tissues, delayed leaf senescence and special root structures with dense clusters of rootlets and root hair (Delgado et al., 2014; Lambers et al., 2015). In contrast, families of Elaeagnaceae, Leguminosae and Ulmaceae showed high N concentrations partly owing to their symbiotic N-fixing microorganisms (Torrey,

1978). Moreover, our finding that leaf N concentrations of Elaeagnaceae were higher than Leguminosae renovated conceptual understandings and provided new reference for the study on plant-microorganism mutualistic symbiosis.

The variations in leaf N and P stoichiometry on family-level support previous reports that leaf N and P concentrations and N:P ratios differ significantly across life forms and the GRH hypothesis: small-size fast-growing herbaceous plants have higher N and P concentrations and lower N:P ratios than large-size slow-growing woodies (Sterner and Elser 2002; Wright et al., 2005; Han et al., 2011; Tian et al., 2018). For example, families of Polygonaceae, Zygophyllaceae, Ranunculaceae and Umbelliferae are mainly herbaceous plants, subshrubs and shrubs, with higher leaf N and P concentrations. Comparatively, families of Myrtaceae, Theaceae, Symplocaceae and Ericaceae with predominately woody species have lower leaf N and P concentrations.

In fact, our results are also in agreement with the biogeochemical niche hypothesis which attributes the differences in plants' key structural, physiological and chemical foliar properties across taxonomic groups to their specific biogeochemical niches (Peñuelas et al., 2010). On the global scale, boreal and temperate biomes are often demonstrated to be N-limited, while tropical ecosystems are P-limited. Hence, leaf N and P stoichiometry of plants distributed in these biomes might be directly influenced by soil nutrient availabilities (Vitousek and Farrington, 1997; Carnicer e al., 2014; Deng et al., 2017). For example, the widespread families of Cupressaceae and Pinaceae in boreal and temperate biomes have low leaf N concentration but comparatively high P concentration, which is a result of the cold environment and soil N limitation. The high leaf P concentrations benefit them for cold resistance and fast growth during their short growth periods (Chapin et al., 1986). On the same rationale, as a majority of Myricaceae, Myrtaceae, Lecythidaceae, Sapotaceae, Symplocaceae, Melastomataceae, Theaceae, Myrsinaceae and Burseraceae families are evergreen woody plants distributing in tropical and subtropical areas, their leaf P concentrations are consequently quite low (i.e. all < 1.00 mg g^{-1}).

4.2 Biogeographic pattern of leaf N and P stoichiometry on family-level

Previous explorations of leaf N and P stoichiometry at regional and global scales have reported the general biogeographic pattern that leaf N and P concentrations decrease but N:P ratios increase with increasing MAT (Reich and Oleksyn, 2004; Han et al., 2005, 2011; Zhang et al.,

2012). Several studies have tried to further quantify the evolutionary and environmental control on variations of leaf element stoichiometry (Watanabe et al., 2007; Zhang et al., 2012; Yang et al., 2017). For example, Watanabe et al. (2007) addressed that over 25% of the total variation in leaf element composition could be explained by family level taxonomy and the remaining variations could be substantially assigned to climatic factors, soil conditions, sampling techniques and differences between species within families. In addition, plant taxonomy largely influences biogeographic patterns of leaf N and P stoichiometry across environmental gradients (i.e. latitude, longitude, altitude, MAT, MAP) (He et al., 2010; Chen et al., 2013; Zhang et al., 2012; Yang et al., 2015), which might imply that leaf N and P stoichiometry is phylogenetically conserved (Sardans and Peñuelas, 2014; Hao et al., 2015; Yang et al., 2015; Sardans et al., 2016). Although our focus in the current study is not on detecting the phylogenetic signal and the convergence of leaf N and P stoichiometry on family-level, our results correspondingly revel that leaf N and P concentrations and N:P ratios differ significantly (Table 1) and show divergent trends along MAT and MAP across families (Figure 1).

For leaf N concentrations, approximately a half families (i.e. 33 of 62 families) declined with increasing MAT and less than 1/3 families (i.e. 22 of 62 families) decreased with increasing MAP, which were in consistent with the general patterns of pooled data (Reich and Oleksyn, 2004; Han et al., 2005). However, the other 29 and 30 families in our dataset showed the opposite trends or no significant change along MAT and MAP, respectively. For leaf P, more than a half families (35 of 62 families) declined with increasing MAP, whereas approximately a half families (30 of 62 families) did not change with MAT. These results resonated with previous generalization on reginal scales that MAT and MAP showed comparatively stronger effects on leaf N and P concentrations, respectively. We additionally attributed some families' lack of pattern along MAT and MAP to their smaller geographic distributions and environmental gradients, such as Symplocaceae, Sapindaceae and Gleicheniaceae families widespread in tropical and subtropical areas (Table S3). Furthermore, the nonsignificant correlation might also imply that leaf N and P stoichiometry of these families are highly conserved with low stoichiometric plasticity due to their narrow geographical and biogeochemical niches (Yu et al., 2010, 2015; Sardans et al., 2016; Guo et al., 2017).

In particular, we amazedly recognized that leaf N:P ratios of 30 and 36 families showed no trends along MAT and MAP, respectively, which was inconsistent with the general pattern that global leaf N:P ratios increased with increasing MAT (Reich and Oleksyn, 2004; Kerkhoff et

al., 2005; Zhang et al., 2018). However, our results resonated with previous studies conducted by Han et al. (2005), Zhang et al. (2012) and Yang et al. (2015). Although it's an open questions whether phylogeny (or genotype) is more important than environment when researchers seek to disentangle the effects of genotypic and environmental factors on leaf N and P stoichiometry, our study highlight the importance and necessity of considering phylogenetic and taxonomic information in plant stoichiometry studies.

4.3 Inconstant leaf N~P scaling relationships on family-level

Our results showed that leaf N and P concentrations of most higher plants coupled significantly with scaling exponents ranging from 0.307 to 0.991, which reinforced the inconstant leaf N~P scaling relationships proposed by Tian et al. (2018) from family-level study. Moreover, the specific exponents of family-level leaf N~P scaling relationships varied substantially with previous overall results (e.g. Wright et al., 2004; Niklas & Cobb 2005; Niklas 2006; Kerkhoff et al., 2006; McGroddy et al., 2008; Reich et al., 2010; Zhao et al., 2016). In addition, the specific exponents of family-level leaf N~P scaling relationships have no correlation with their sample sizes (Figure S4 a and b), implying that family-level leaf N~P scaling exponents might not consequentially converge to a certain value with their increasing sample sizes.

As a part of leaf economic spectrum (Wright et al., 2004), 'conserved' leaf N~P scaling relationship is the key determining its practical application. For example, Price et al. (2014) examined the 'invariance' of LES and concluded that all LES traits approached invariance within and between plant lifeforms, taxonomic groups and biomes. However, our results displayed inconsistency that leaf N~P scaling relationship varied significantly even in the same life from. For example, leaf N~P scaling exponents of Myricaceae and Tamaricaceae were the highest (i.e. 0.965) and the lowest (i.e. 0.307), respectively, among 62 families, even though they are both in woody groups.

Kerkhoff et al. (2006) ascribed their find of strong phylogenetic signals and similar scaling relationships of leaf N and P concentrations to the influences of both evolutionary history and environmental molding. Nevertheless, our results presented large variations of leaf N and P stoichiometry across different families of higher plants, reasserting our recent statement that the canonical numerical value of global leaf N~P scaling exponent might be a result of pooled data analysis which hides or neglects biologically and ecologically significant variations (Tian et al., 2018). Actually, no canonical leaf N~P scaling relationship probably hold true across all

plant lineages. Here, for pooled data, the mutual offsets among different families might also produce a certain numeric value that misinterpret their inherent stoichiometric relationships. For example, as illustrated in Figure S4 a and b, leaf N~P scaling exponent generally, but not all (e.g. Compositae), approached 2/3 for families with large sample sizes (e.g. Leguminosae, Gramineae). Hence, it remains difficult to distinguish whether the 2/3-power N~P scaling relationship is a result of the differences among species and individuals within the same family or the true stoichiometric pattern of the family? Clearly, statistic analysis from pooled data could not bring out an irrefutable truth.

5 CONCLUSION

Through establishing the largest global leaf N and P concentration dataset of higher plants, we analyzed leaf N and P stoichiometric patterns of specific families. Our results demonstrated that large variations existed in leaf N and P concentrations, N:P ratios and N~P scaling relationships across different families, which further extended leaf N and P stoichiometry across life-forms and ecological biomes reported by Tian et al. (2018). Family-level geographical patterns of leaf N and P stoichiometric relationships along MAT and MAP gradients and inconstant N~P scaling exponents revealed the imperative needs to incorporate phylogenetic and taxonomic groupings in plant stoichiometry studies. Comparing with rough groupings of angiosperm and gymnosperm or plant life forms (i.e. functional groups), our family-level results of leaf nutrient stoichiometry could avoid some errors originated from pooled data and provide useful parameters for large-scale ecological models.

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Table 1. Leaf N and P stoichiometry of 62 families in our study. *n* represents the sample size of each family.

Family	*n*	N (mg g^{-1})		P (mg g^{-1})		N:P	
		Geometric mean	SE	Geometric mean	SE	Geometric mean	SE
Aceraceae	94	22.45	0.54	1.86	0.07	12.06	0.42
Anacardiaceae	107	16.59	0.61	1.21	0.06	13.76	0.53
Annonaceae	56	21.32	0.69	1.00	0.06	21.29	1.29
Apocynaceae	38	20.01	0.95	1.12	0.09	17.85	1.22
Aquifoliaceae	64	15.12	0.43	0.70	0.03	21.55	0.83
Araliaceae	79	21.92	1.01	1.64	0.10	13.34	0.48
Berberidaceae	43	18.34	0.79	1.24	0.08	14.78	1.03
Betulaceae	323	22.50	0.33	1.67	0.04	13.48	0.29
Burseraceae	70	16.45	0.42	0.72	0.03	23.02	1.08
Caprifoliaceae	237	19.36	0.48	1.45	0.04	13.37	0.33
Caryophyllaceae	35	21.00	1.17	1.69	0.19	12.46	1.55
Celastraceae	131	22.57	0.60	1.54	0.06	14.65	0.46
Chenopodiaceae	173	23.44	0.66	1.48	0.07	16.04	0.71
Compositae	1061	16.88	0.21	1.45	0.03	11.60	0.19
Coriariaceae	37	14.84	1.24	1.10	0.07	13.46	1.24
Cupressaceae	62	10.89	0.37	1.04	0.06	10.49	0.52
Cyperaceae	314	18.67	0.36	1.30	0.04	14.45	0.40
Elaeagnaceae	42	30.30	1.05	1.51	0.13	20.01	1.30
Ericaceae	499	14.32	0.19	0.98	0.02	14.63	0.27
Eucommiaceae	157	20.18	0.60	1.18	0.05	17.34	0.74
Euphorbiaceae	54	16.82	0.82	1.31	0.10	12.87	0.87
Fagaceae	480	17.89	0.24	1.06	0.02	16.96	0.32
Gleicheniaceae	57	10.08	0.40	0.43	0.03	23.38	1.44
Gramineae	1019	18.53	0.23	1.35	0.03	13.74	0.23
Guttiferae	35	15.04	0.92	0.88	0.08	17.03	1.42
Hamamelidaceae	98	13.95	0.33	0.89	0.03	15.70	0.53
Juglandaceae	64	19.44	0.88	1.23	0.10	15.81	0.93
Labiatae	111	21.87	0.70	1.80	0.08	12.15	0.49
Lauraceae	163	18.49	0.49	0.90	0.03	20.49	0.74
Lecythidaceae	81	20.51	0.54	0.64	0.02	31.83	1.10
Leguminosae	1122	27.04	0.29	1.20	0.02	22.57	0.27
Liliaceae	75	24.40	1.18	1.81	0.12	13.47	0.65
Melastomataceae	82	14.87	0.52	0.66	0.03	22.44	0.74
Meliaceae	35	19.51	0.97	1.48	0.14	13.15	1.11
Moraceae	145	22.64	0.72	1.19	0.06	19.01	1.68
Myricaceae	41	16.82	0.29	0.48	0.03	35.40	1.73
Myrsinaceae	79	14.81	0.50	0.71	0.04	20.73	1.14
Myrtaceae	222	10.81	0.19	0.55	0.02	19.75	0.43

Oleaceae	147	21.42	0.54	1.39	0.06	15.38	0.55
Pinaceae	301	13.52	0.21	1.24	0.04	10.91	0.29
Plantaginaceae	50	16.79	0.92	1.53	0.16	10.95	0.88
Polygonaceae	97	28.09	1.01	1.98	0.14	14.22	0.80
Primulaceae	50	18.36	1.07	1.46	0.13	12.70	0.81
Proteaceae	198	6.11	0.16	0.27	0.01	22.53	0.63
Ranunculaceae	66	25.35	0.84	1.95	0.14	13.03	0.96
Rhamnaceae	162	21.90	0.52	1.19	0.04	18.42	0.50
Rosaceae	1008	19.26	0.19	1.47	0.02	13.12	0.19
Rubiaceae	209	17.83	0.43	0.86	0.03	20.83	0.74
Rutaceae	107	23.18	0.66	1.21	0.07	19.11	0.86
Salicaceae	312	21.57	0.41	1.80	0.05	11.97	0.30
Sapindaceae	46	18.76	0.71	1.28	0.10	14.68	1.03
Sapotaceae	78	15.23	0.62	0.65	0.03	23.58	1.27
Saxifragaceae	161	21.75	0.51	1.69	0.07	12.91	0.50
Scrophulariaceae	50	20.42	0.65	1.59	0.13	12.87	1.08
Symplocaceae	58	14.72	0.36	0.66	0.03	22.21	0.90
Tamaricaceae	67	18.66	1.20	0.88	0.06	21.32	0.98
Theaceae	175	13.10	0.32	0.68	0.02	19.29	0.56
Tiliaceae	67	24.95	0.85	1.83	0.07	13.61	0.60
Ulmaceae	133	23.93	0.53	1.52	0.06	15.74	0.59
Umbelliferae	57	24.40	0.90	2.12	0.16	11.50	0.81
Verbenaceae	203	21.17	0.52	1.31	0.03	16.12	0.44
Zygophyllaceae	60	29.40	1.37	1.42	0.08	20.69	0.82

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Table 2. Exponents of reduced major axis (RMA) regression between leaf N and leaf P concentrations of 54 families, e.g. $\log_{10}$ leaf N = α $\log_{10}$ leaf P + $\log_{10}$ β , and the comparisons with 2/3 and 3/4, respectively. " √ " indicates that the exponent of leaf N vs. leaf P scaling is not statistically significantly different from 2/3 or 3/4; "×" indicates that the exponents of leaf N vs. leaf P scaling are lower or higher than 2/3 or 3/4, respectively. The *p* values are summarized from a likelihood ratios test.

Family	α_{RMA} (95% CI)	Compare with 2/3		Compare with 3/4	
		2/3	*p*	3/4	*p*
Aceraceae	0.631 (0.525, 0.759)	√	0.559	√	0.067
Anacardiaceae	0.756 (0.650, 0.880)	√	0.104	√	0.919
Apocynaceae	0.594 (0.447, 0.789)	√	0.418	√	0.105
Aquifoliaceae	0.683 (0.545, 0.857)	√	0.812	√	0.417
Araliaceae	0.777 (0.676, 0.891)	×	0.031	√	0.619
Betulaceae	0.656 (0.592, 0.727)	√	0.823	×	0.013
Caprifoliaceae	0.859 (0.775, 0.953)	×	<0.001	×	0.010
Celastraceae	0.640 (0.561, 0.730)	√	0.534	×	0.018
Chenopodiaceae	0.594 (0.515, 0.684)	√	0.106	×	0.001
Compositae	0.628 (0.597, 0.660)	×	<0.001	×	<0.001
Cupressaceae	0.576 (0.464, 0.716)	√	0.187	×	0.018
Cyperaceae	0.596 (0.542, 0.656)	×	0.022	×	<0.001
Elaeagnaceae	0.420 (0.331, 0.531)	×	0.003	×	<0.001
Ericaceae	0.581 (0.541, 0.623)	×	<0.001	×	<0.001
Eucommiaceae	0.752 (0.644, 0.879)	√	0.127	√	0.969
Euphorbiaceae	0.619 (0.488, 0.784)	√	0.531	√	0.109
Fagaceae	0.649 (0.598, 0.704)	√	0.523	×	<0.001
Gleicheniaceae	0.650 (0.505, 0.837)	√	0.859	√	0.277
Gramineae	0.666 (0.630, 0.703)	√	0.952	×	<0.001
Guttiferae	0.663 (0.487, 0.902)	√	0.969	√	0.426
Hamamelidaceae	0.666 (0.554, 0.801)	√	0.992	√	0.206
Juglandaceae	0.574 (0.476, 0.692)	√	0.115	×	0.006
Labiatae	0.679 (0.578, 0.797)	√	0.826	√	0.221
Lauraceae	0.724 (0.627, 0.834)	√	0.260	√	0.620
Lecythidaceae	0.836 (0.676, 1.034)	×	0.038	√	0.318
Leguminosae	0.689 (0.658, 0.721)	√	0.169	×	<0.001
Liliaceae	0.762 (0.639, 0.908)	√	0.135	√	0.860
Melastomataceae	0.768 (0.656, 0.898)	√	0.063	√	0.694
Meliaceae	0.527 (0.386, 0.718)	√	0.134	×	0.026
Moraceae	0.688 (0.607, 0.781)	√	0.621	√	0.181
Myricaceae	0.307 (0.233, 0.404)	×	<0.001	×	<0.001
Myrsinaceae	0.641 (0.516, 0.797)	√	0.725	√	0.157
Myrtaceae	0.609 (0.550, 0.675)	√	0.087	×	<0.001
Oleaceae	0.621 (0.539, 0.716)	√	0.368	×	0.012

Pinaceae	0.507 (0.459, 0.559)	×	<0.001	×	<0.001
Plantaginaceae	0.536 (0.427, 0.672)	√	0.058	×	<0.001
Polygonaceae	0.509 (0.434, 0.597)	×	0.001	×	<0.001
Primulaceae	0.673 (0.544, 0.731)	√	0.934	√	0.309
Proteaceae	0.770 (0.687, 0.862)	×	0.014	√	0.655
Rhamnaceae	0.711 (0.628, 0.805)	√	0.307	√	0.410
Rosaceae	0.613 (0.580, 0.648)	×	0.003	×	<0.001
Rubiaceae	0.599 (0.532, 0.676)	√	0.081	×	<0.001
Rutaceae	0.527 (0.450, 0.618)	×	0.004	×	<0.001
Salicaceae	0.738 (0.666, 0.817)	×	0.052	√	0.749
Sapindaceae	0.491 (0.374, 0.644)	×	0.028	×	0.003
Saxifragaceae	0.533 (0.465, 0.611)	×	0.001	×	<0.001
Symplocaceae	0.575 (0.448, 0.737)	√	0.241	×	0.036
Tamaricaceae	0.991 (0.841, 1.167)	×	<0.001	×	0.001
Theaceae	0.846 (0.740, 0.967)	×	<0.001	√	0.084
Tiliaceae	0.875 (0.691, 1.108)	×	0.024	√	0.199
Ulmaceae	0.578 (0.492, 0.680)	√	0.083	×	0.002
Umbelliferae	0.487 (0.381, 0.622)	×	<0.001	×	0.001
Verbenaceae	0.949 (0.837, 1.076)	×	<0.001	×	<0.001
Zygophyllaceae	0.859 (0.713, 1.035)	×	0.008	√	0.147

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Figure 1. Relationships between leaf N and P stoichiometry on family-level and MAT and MAP, respectively. (a) leaf N concentration against MAT; (b) leaf P concentration against MAT; (c) leaf N:P ratios against MAT; (d) leaf N against MAP; (e) leaf P against MAP; (f) leaf N:P ratios against MAP. Note: each color indicates a family. Each data point denotes an individual record. The solid lines indicate significant correlations with $p<0.05$. MAT, mean annual temperature. MAP, mean annual precipitation.

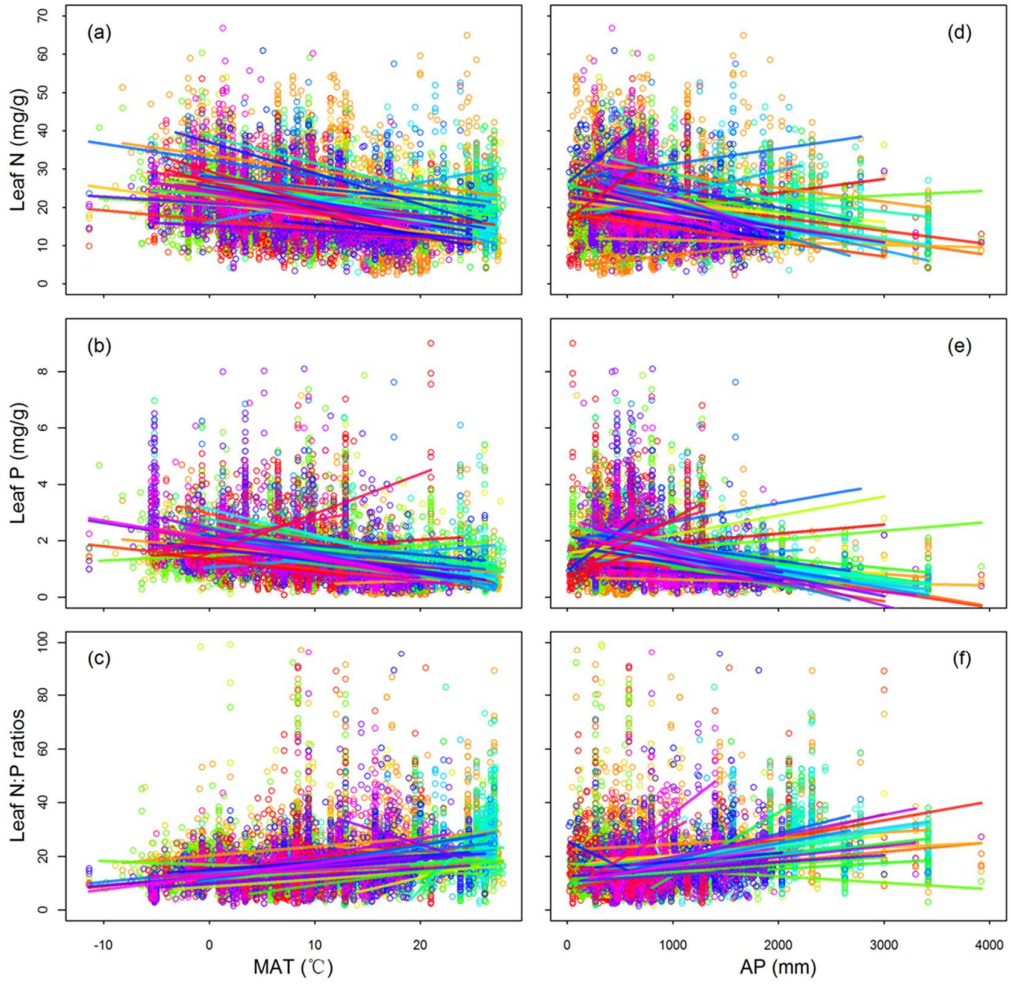
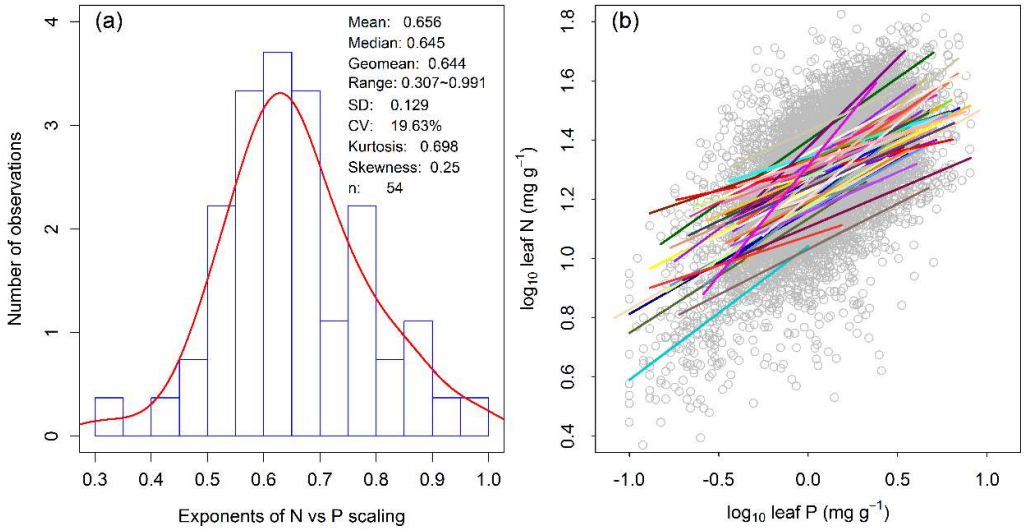


Figure 2. The leaf N and leaf P scaling relationships of 54 families from our dataset. (a) Frequency distribution of the scaling exponents of 54 families; (b) Relationships between leaf N and leaf P concentrations of each family. The solid line in each color in (b) indicate the significant leaf N~P scaling relationship on family-level with $p<0.05$. Scaling exponents were calculated from the RMA regression between leaf N and leaf P concentrations. e.g., log10 leaf N = α log10 leaf P + log10 β.



SUPPORTING INFORMATION

Table S1. The statistics including geometric means, ranges, coefficient of variance (CV), standard error (SE) of leaf N and P concentrations, N:P ratios and N~P scaling relationships based on the randomly sampled records using Monte Carlo methods.

Table S2. The statistics of leaf N and P scaling exponents calculated from the randomly sampled records using Monte Carlo methods by the RMA regression between leaf N and leaf P concentrations, e.g., log10 leaf N = α log10 leaf P + log10 β, including *p*-values, r^2, 95% CI of the slopes and constants. Each regression relationships were statistically significant with $p<0.05$. NA represents nonsignificant relationships with $p>0.05$.

Table S3. The ranges of 62 families' distributions including ranges of latitude, MAT and MAP, and the regression analysis results of leaf N and P concentrations and N:P ratios against MAT and MAP. Each regression relationships were statistically significant with $p<0.05$. NA represents nonsignificant relationships with $p>0.05$.

Figure S1. Leaf N and P concentrations of different sampling sizes by Monte Carlo Sampling. (a) and (b) indicate leaf N concentration from 0 to 100 and 100 to 10000, respectively. (c) and (d) indicate leaf P concentration from 0 to 100 and 100 to 10000, respectively. The geometric mean values of leaf N and P concentrations were showed.

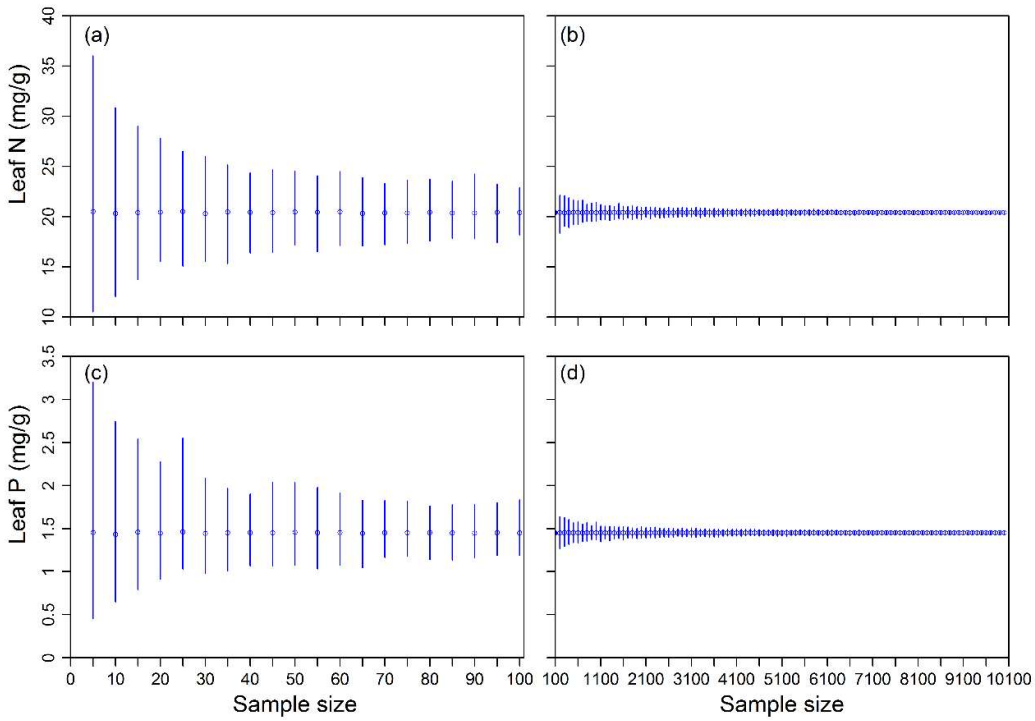
Figure S2. Leaf N and P ratios and N~P scaling exponents of different sampling sizes by Monte Carlo Sampling. (a) and (b) indicate leaf N:P ratios from 0 to 100 and 100 to 10000, respectively. (c) and (d) indicate leaf N~P scaling exponents from 0 to 100 and 100 to 10000, respectively. Reduced major axis (RMA) regression was used to determine the N vs. P scaling relationship. The geometric mean values of leaf N:P ratios and leaf N~P scaling exponents were showed.

Figure S3. Statistics of N~P scaling exponents of different sampling sizes by Monte Carlo Sampling. (a) shows the numbers of significant N~P scaling (with $p<0.05$) in each 1000 runs during sampling of different sample sizes; (b) indicates the CVs of N~P scaling exponents of in each 1000 runs during sampling of different sample sizes. Reduced major axis (RMA) regression was used to determine the N vs. P scaling relationship.

Figure S4. Relationships between family-level N vs P scaling exponent and (a) the specific

624 sample size of a family; and (b) number of species within a family. The red dashed line
625 represents the empirical values of 2/3.

626 Figure S1.



627 Figure S2.

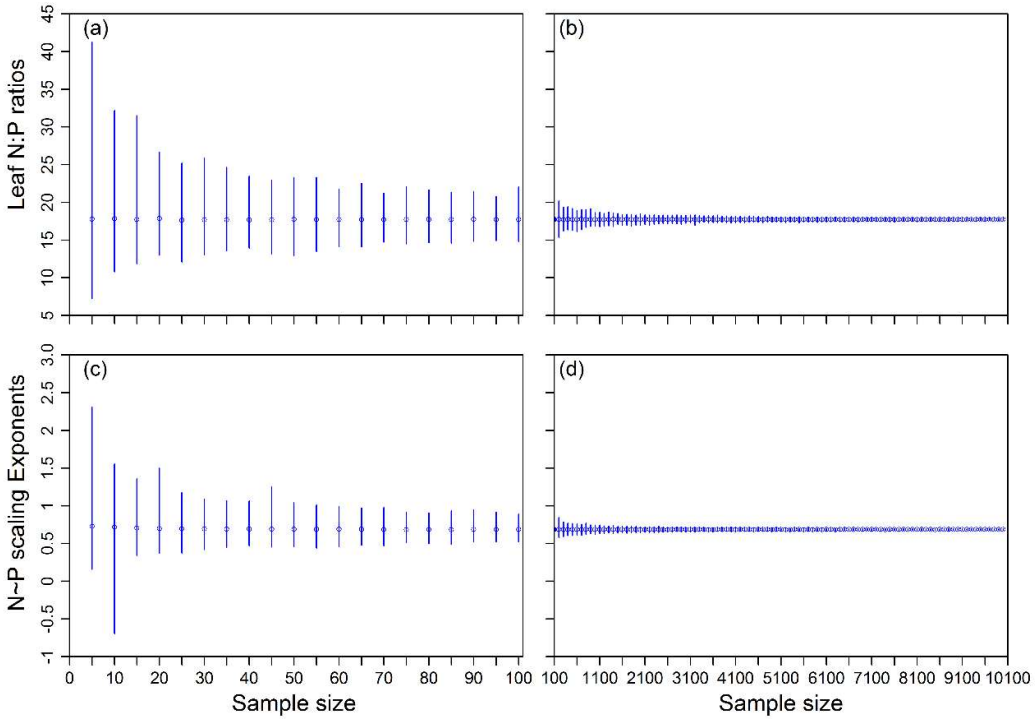


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Figure S3.

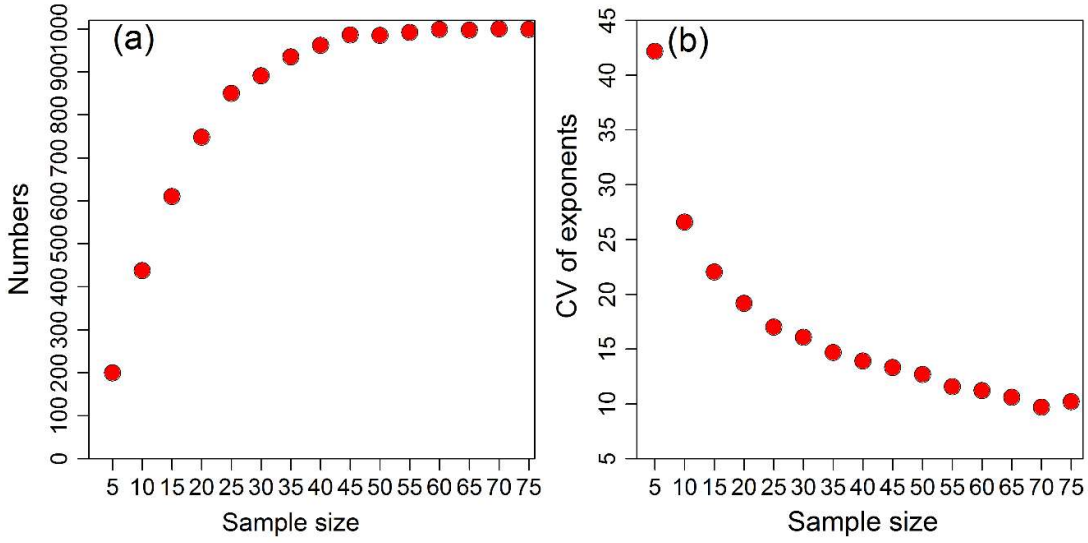
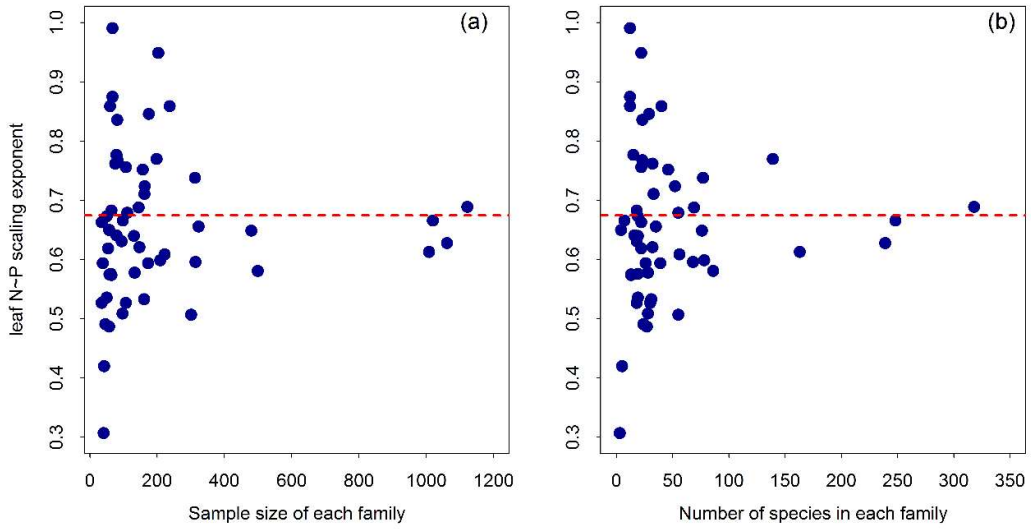

(a)
Numbers
0 100 200 300 400 500 600 700 800 900 1000
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Sample size
(b)
CV of exponents
5 10 15 20 25 30 35 40 45
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Sample size

Figure S4


(a)
(b)
leaf N~P scaling exponent
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
200
400
600
800
1000
1200
Sample size of each family
0
50
100
150
200
250
300
350
Number of species in each family